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DE PLANTIS TOXICARIIS E MUNDO NOVO TROPICALE COMMENTATIONES XVIII

Phytochemical examination of Spruce's ethnobotanical collection of *Anadenanthera peregrina*.

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One of the classical hallucinogens of the Americas is the snuff prepared from beans of the leguminous tree *Anadenanthera peregrina* (L.)Speg., better known in the literature by its former name *Piptadenia peregrina* (L.)Benth. (1).

Long known from the Orinoco River basin of Colombia and Venezuela, this psychoactive drug has been mentioned by virtually all of the early scientific explorers of the area. In 1916, it was identified by Safford as the source of the enigmatic *cohoba*, the narcotic snuff of the West Indies, the use and effects of which were seen among the Taino Indians of Hispaniola by early Spanish explorers in 1496 (2).

While the drug is no longer employed anywhere in the Caribbean islands, the extent of the use of *Anadenanthera peregrina* has still not been clearly defined. It may be that, in isolated localities in the southern part of the Amazon Valley, the tree was until recently the source of a snuff. There is circumstantial evidence, too, that the very closely allied *Anadenanthera co-*

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lubrina was employed in preparing an intoxicating snuff known as *cébil* or *huilca*, used in former times in parts of northern Argentina, Paraguay and possibly in Bolivia and Peru (3).

Our earliest botanical knowledge of *niopo* or *yopo* — as the snuff is called in the Orinoco — goes back to 1801, when von Humboldt and Bonpland encountered its use in Colombia and Venezuela (4). Kunth reported briefly on their observations: "Ex seminibus tritis calci vivae admixtis fit tabacum nobile quo Indi Otomacos et Guajibos utuuntur." Humboldt identified the source as *Piptadenia Niopo*, which he believed represented the same species as Willdenow's *Inga Niopo*. Humboldt, like the earlier explorer of the Orinoco, Padre Gumilla, erroneously believed that the intoxicating effects of the snuff could be attributed to the alkaline admixture and not to the seeds employed in elaborating the powder.

The next major botanical encounter with the drug was that of Richard Spruce, who met with its use in June, 1854 amongst the Guahibos of the upper Orinoco (5). Spruce wrote that his "specimens of the leaves, flowers and fruit agree so well with Kunth's description of *Acacia Niopo* that I cannot doubt their being the same species; especially as I have traced the tree all the way from the Amazon to the Orinoco, and found it everywhere identical." An important point in Spruce's meticulous observation of the preparation of the snuff, however, is his statement that "there is no admixture of quicklime."

Spruce found ". . .a wandering horde of Guahibo Indians . . . encamped on the svannas of Maypures [on the Orinoco] and . . . an old man grinding Niopo seeds . . . . The seeds, being first roasted, are powdered on a wooden platter, nearly the shape of a watch-glass, but rather longer than broad (9¼ inches by 8 inches). It is held on the knee by a broad, thin handle, which is grasped in the left hand, while the fingers of the right hold a small spatula or pestle of the hard wood of the Palo de arco (*Tecoma* sp.) with which the seeds are crushed . . . . For taking the snuff, they use an apparatus made of the leg-bones of herons . . . in the shape of the letter Y, or something like a tuning-fork, and the two upper tubes are tipped with small black perforated knobs (the endocarps of a palm). The lower tube being inserted in the snuff-box and the knobs in the nostrils, the snuff is forcibly inhaled, with the effect of thoroughly narcotising a novice or indeed a practiced hand, if taken in sufficient quantity . . . ." The apparatus which Spruce described and which he purchased at Maypures on the Orinoco may still be seen at the Royal Botanic Gardens at Kew. An illustration of this parephernalia is herewith published.

There are also at Kew — in the Economic Botany Museum — specimens of the pods of *Anadenanthera peregrina* which Spruce collected in 1854 on the Colombo-Venezuelan border at the Savannahs of Maypures. These pods were purchased from an old Guahibo Indian who was grinding the seeds for preparation of the snuff.

In our desire to analyze as many specimens of seeds of this species from as many localities as possible, we expressed to the authorities at Kew our interest in submitting some of Spruce's 120-year old material to modern chemical examination. We owe a debt of gratitude to the former director, Dr. John Heslop-Harrison, the former Keeper, Dr. J. P. M. Brenan, Dr. Tony Swain, formerly director of the Biochemical Laboratory and Miss Rosemary Angel of the Economic Botany Museum for finding and making available to us the necessary pods and seeds. The analytic study is detailed below.

We were encouraged to examine this important material collected by Spruce for several reasons (6,7).

First: we wanted to compare its analysis with that of very recently collected material.

Second: Spruce was far ahead of the customs of botanical explorers of his time in being willing to collect material of medicinal and narcotic plants for chemical analysis.

Third: we had been successful in analyzing material of the hallucinogen *Banisteriopsis Caapi* of the Malpighiaceae, collected by Spruce on the Rio Uaupés of Brazil in 1852 (8). This material was examined in April 1968 in the Karolinska Institutet in Stockholm, 114 years after its collection. The yield of alkaloids was 0.4% as against 0.5% for a recently collected specimen of the same species. The alkaloid content of Spruce's material consisted exclusively of harmine, as contrasted with harmine, harmaline and tetrahydroharmine, as well as two minor constituents in the modern material.

As we did with the Spruce material of *Banisteriopsis*, the examination of the seeds of the collection *Spruce 119* of *Anadenanthera peregrina* was compared with the analysis of similar freshly collected material. For several years, we have been studying a colony of beautiful trees of this species — obviously planted, perhaps some 40 years ago — in Barrio St. Just of Carolina, near San Juan, Puerto Rico. This colony grows on a hill immediately behind the El Comandante horse-racing track. Our most recent botanical studies on the colony were made in the month of December 1974, when the pods are still immature. Mature pods for the present analyses were also collected from the same colony of trees by Dr. Thomas Schubert and Mr. José Zambrana of the United States Department of Agriculture Forest Service, Institute of Tropical Forestry, Río Piedras, Puerto Rico on March 13, 1975, when the pods had fully ripened.

All of our collections from the Puerto Rican site are deposited in the Economic Herbarium of Oakes Ames in the Harvard Botanical Museum.

PUERTO RICO: La Carolina, Barrio St. Just, adjacent to Hipódromo. "Tree 60 feet tall. Pods brownish. *Cojoba*." December 8, 1970. *R. E. Schultes 26091*. — Same locality and date. "Seedlings under tree of collection *26091*." *R. E. Schultes 26091 A*. — Same locality. "Tree in grove on hillside. Height 70 feet. Pods green-brown, ripening black. Bark with large conical spines." December 13, 1972. *R. E. Schultes 26363*. — Same locality and date. *R. E. Schultes 26364*. — Same locality. "Tree 45 feet tall. Secondary forest. Cork black." December 12, 1974. *R. E. Schultes, S. von R. Altschul et B. Holmstedt s.n.*

CHEMICAL ANALYSIS

MATERIAL AND METHODS: The following botanical materials will be referred to as below:

seeds "December 1972"— for immature seeds collected in Puerto Rico. Voucher specimen: *R. E. Schultes 26363*, December 13, 1972; and

seeds "March 1975"— for mature seeds collected from the same colony of trees in Puerto Rico. Voucher specimen: *R. E. Schultes, S. von R. Altschul et B. Holmstedt s.n.*, December 12, 1974.

REFERENCE SUBSTANCES: All reference substances have been previously described (9).

ABBREVIATIONS:

DMT	N,N-dimethyltryptamine
5-MeO-DMT	5-methoxy-N,N-dimethyltryptamine
5-MeO-MMT	5-methoxy-N,N-monomethyltriptamine
5-OH-DMT	5-hydroxy-N,N-dimethyltryptamine or bufotenine
MTHC	2-methyl-1,2,3,4-tetrahydro-β-carboline
6-MeO-THC	2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline
6-MeO-DMTHC	1,2-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β--carboline

ISOLATION OF THE ALKALOIDS: The vegetal material was ground and extracted according to a procedure first used by Fish *et al.* (10).

GAS CHROMATOGRAPHY (GC): The gas chromatographic analyses were performed with a Varian Model 2100 GC equipped with a flame ionization detector system. A 180 x 0.2 cm (i.d.) glass column was silanized and packed with 3% OV-17 coated on Gas Chrom Q, 100-120 mesh (Applied Science Laboratories, State Coll., Pa.). The separations were obtained at a column temperature of 190°C with a nitrogen carrier gas flow rate of 30 ml per min. The vaporizer and the detector temperatures were 250°C and 300°C, respectively. The amounts of alkaloids were determined by peak heights using 5-hydroxy-N, N-dimethyltryptamine as a standard.

GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC-MS): An LKB Model 9000 GC-MS (LKB-Produkter AB, Bromma, Sweden) was used to confirm the structure of the alkaloids. Separation was obtained on a 160 x 0.2 cm (i.d.) silanized glass column, packed with the same packing material as for the GC analyses but maintained at 170°C. The flow rate of the helium carrier gas was 40 ml per min. The ionizing potential and the trap current were 70 eV and 60 μA, respectively. The ion source was kept at 250°C.

Table 1. Gas chromatographic and mass spectrometric data for reference compounds.

Compound[a]	R_t[b]	Mass spectrum[c]
DMT	2.5	58 (base peak), 103, 105, 130, 143, 188 (M^+)
MTHC	4.3	78, 102, 115, 143 (base peak), 186 (M^+)
5-MeO-DMT	5.8	58 (base peak), 103, 117. 160, 173, 218 (M^+)
5-OH-DMT	8.4	58 (base peak), 103, 117, 146, 159, 204 (M^+)

[a]For abbreviations, see material and methods.

[b]LKB 9000 GC-MS with helium as carrier gas on 3% OV-17 on Gas Chrom Q at 170°C. R_t= retention time in minutes.

[c]Ionizing potential was 70 eV. m/e values of the major peaks are given.

MASS FRAGMENTOGRAPHY (MF): In order to confirm the presence or absence of minor alkaloids in the plant materials, the specific and sensitive method of mass fragmentography was used (11). The principles of the technique have already been described (12). The mass spectrometer was controlled by a PDP-12 computer system. The channels used were focussed carefully on the molecular ion of each compound of interest: m/e = 204 for 5-OH-DMT; m/e = 186 for MTHC; m/e = 188 for DMT; and m/e = 218 for 5-MeO-DMT. During another experiment m/e = 58 was chosen with two different sensitivities on two channels.

RESULTS

The gas chromatographic trace of the chloroform-soluble bases from the seeds collected in 1854 by Spruce gave a single peak. Its mass spectrum was identical to that of 5-OH-DMT. The mass fragmentogram of the same extract is in agreement with that result (Fig. 1). The extract of mature seeds freshly collected in Puerto Rico, seeds "March 1975", showed several GC peaks. Beside 5-OH-DMT they have been identified by GC-MS and are DMT, MTHC and 5-MeO-DMT (Table 1). The mass fragmentographic recording confirms in a single run the presence of the four alkaloids (Fig. 2). The relative amount of the compounds in the plant material is given in Table 2. In the same table are listed the results of similar analyses of various plant parts of *Anadenanthera peregrina*, originating from the same colony of trees in Puerto Rico. Included in the table are also specimens of more or less well defined botanical or ethnological origin.

DISCUSSION

The finding of 5-OH-DMT as the only alkaloid in the Spruce material is significant for several reasons. First: it indicates that, with modern analytical tools, it is possible to detect and identify alkaloids in plant materials more than 100 years old. Second: identification of the botanical specimen is strengthened by the results of the chemical analyses, because the same compound has been found in both old and the freshly collected seeds. One previous analysis of seeds of *Anadenanthera peregrina* originating from Puerto Rico has shown the presence of 5-OH-DMT as the principal alkaloid (13). A sample of *A. peregrina* seeds collected in southern Venezuela contained 7.5% of 5-OH-DMT (14, 15). Holmstedt and Lindgren (16) have reviewed the alkaloid composition of many specimens.

DMT alone or together with 5-MeO-DMT has been isolated from *Anadenanthera peregrina* originating from Brazil (10). A similar composition was found in related species (9, 16).

Table 2 illustrates the differences in alkaloid contents in various parts of *Anadenanthera peregrina*. The root contained the highest amount of alkaloids. In this collection, 5-MeO-DMT was the predominant alkaloid in all plant parts, except the seeds, where DMT was found in the highest amount. However, it should be mentioned here that the seeds were not fully ripe at the time of collection (seeds "December 1972").

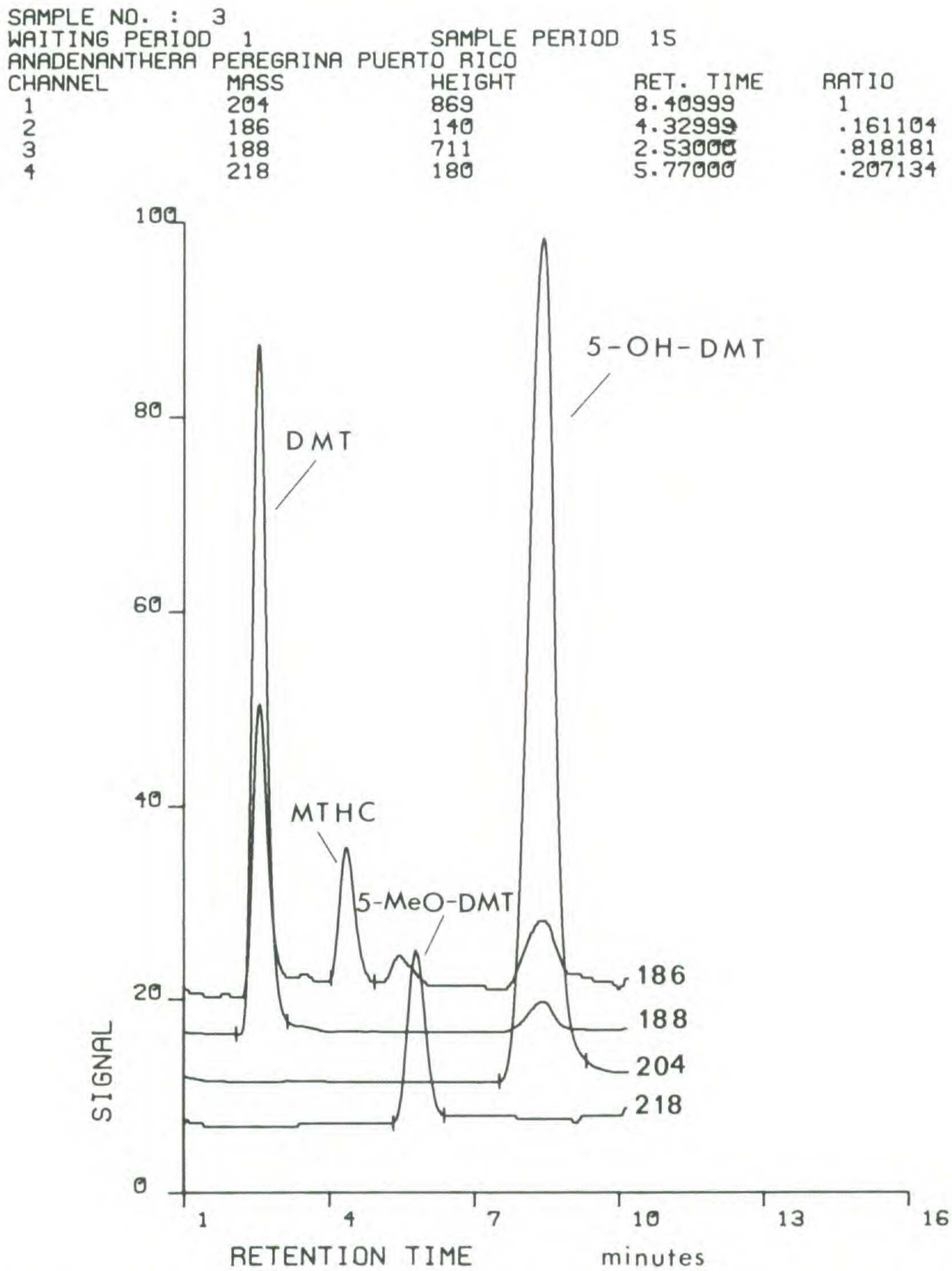

Fig. 1. Mass fragmentogram (OV-17) of the alkaloidal fraction from the *Anadenanthera peregrina* seeds collected by Richard Spruce in 1854.

Since it may be assumed (in fact, examination of the seeds established) that the seeds collected by Spruce had matured, it was necessary for comparison to analyze similar material. Investigation of mature seeds ("March 1975") from the Puerto Rican locality, where immature seeds had been collected pre-

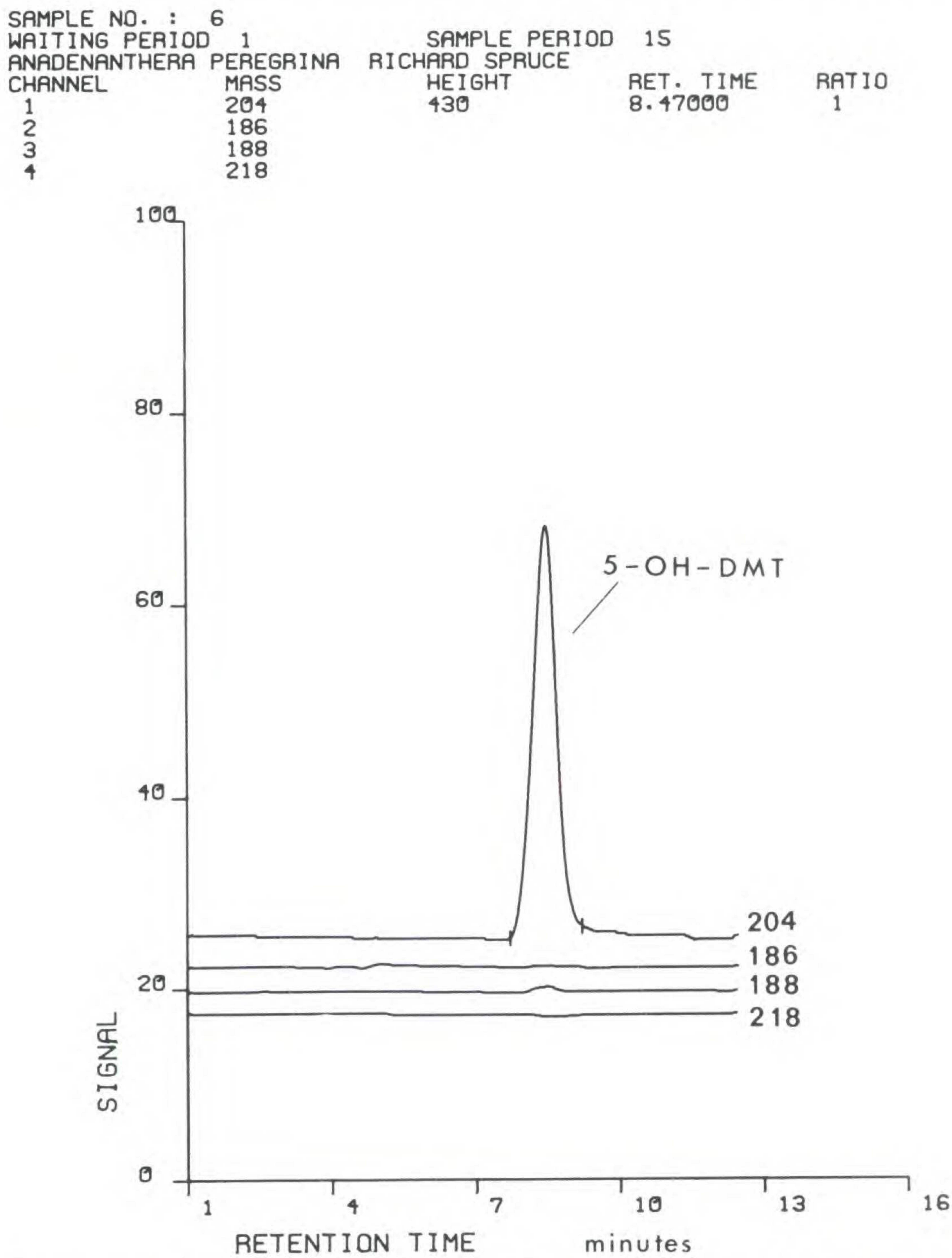

Fig. 2. Mass fragmentogram (OV-17) of the alkaloidal fraction from the *Anadenanthera peregrina* seeds of Puerto Rico (seeds "March 1975") analysed in 1975).

Table 2. Distribution of the indole alkaloids.

Voucher No.	Species	Part of the plant	Alkaloids mg/100 g dry plant	Alkaloids	%
R. Spruce 119	A. peregrina o: Rio Negro, Brazil, 1854	Seeds	614	5-OH-DMT	100
R.E. Schultes, S. von R. Altschul and B. Holmstedt, sin. num	A. peregrina o: San Juan, Puerto Rico, 1975, "March 1975"	Seeds	a) not determined (analysed 1975)	5-OH-DMT DMT 5-MeO-DMT MTHC	80 19 1 traces
			b) 3523 (analysed 1977)	5-OH-DMT	100
R.E. Schultes 26363	A. peregrina o: San Juan, Puerto Rico, 1972, "March 1972"	Seeds	209	DMT 5-MeO-DMT 5-OH-DMT	75 19 6
		Seedlings	25	DMT 5-MeO-DMT 5-OH-DMT	4 95 1
		Pods without seeds	13	DMT 5-MeO-DMT 5-OH-DMT	8 91 1
		Leaves	107	DMT 5-MeO-DMT	12 88
		Twigs	38	DMT 5-MeO-DMT 5-OH-DMT	5 94 1
		Bark	410	DMT 5-MeO-DMT	5 95
		Roots	699	DMT 5-MeO-DMT 5-OH-DMT	2 97 1
	Anadenanthera presumably c: Biocca Cocco, 1963 o: Upper Orinoco El Platanal Machekototeri	Seeds	1	DMT	100
	Anadenanthera c: Biocca Cocco, 1965 o: Upper Orinoco Rio Ocamo	Seeds	6	DMT	100
	Anadenanthera c: G. Seitz, 1965	Seeds	38	DMT	100
	Anadenanthera c: G. Seitz, 1965	Seedlings	29	DMT 5-MeO-DMT	96 4
R.E. Schultes 24625	A. peregrina o: Boa Vista, Brazil (9)	Leaves	13	DMT 5-MeO-DMT	49 48
		Bark	42	DMT 5-MeO-DMT 6-MeO-DMTHC 5-MeO-MMT 6-MeO-THC	1 59 2 36 2
Abbott Lab., 1948 N2003C	Piptadenia peregrina o: San Juan Puerto Rico, (16)	Seeds	9	DMT	100
	Piptadenia peregrina c: J. Yde, 1964, H4685	Seedlings	1	DMT	100
	Piptadenia, Tupari c: Caspar, 1964 o: Guaporé, Brazil	Seeds	13	DMT 5-MeO-DMT	15 85
	Schupfsnuff, Tupari c: G. Baer, 1964 o: Brazil	Snuff	16	DMT	100
	Yopo c: L. Persson, 1966 o: R. Miriti-Parana Caqueta, Columbia	Snuff	16	DMT	100

c: = collector
o: = origin

viously, shows a different picture [mostly 5-OH-DMT and DMT, with less MTHC and 5-MeO-DMT (Fig. 2)]. Analysis of these seeds was done in August 1975, five months after collection. No quantitation of alkaloid contents was performed at that time.

We repeated the analysis of the same material two years later. At this time, the Puerto Rican seeds were no longer able to germinate. In the analysis of this material, the seeds ("March 1975") contained only 5-OH-DMT, with no trace of any of the other alkaloids found earlier. This fact might imply that the relative content of the various alkaloids upon storage follows with time a certain pattern.

The seeds of the Puerto Rican material kept for two years and the 123 year old Spruce material thus contain the same alkaloid: 5-OH-DMT. Transformation of alkaloids during storage of botanical material is known to occur (17).

CONCLUSIONS

In yet another ethnobotanical collection made by Spruce more than a 100 years ago, it has been possible to identify alkaloidal material by the use of modern analytical techniques never dreamed of by this intrepid plant explorer. Of several alkaloids found in freshly collected reference material, only one remained in the Spruce collection: bufotenine (5-OH-DMT). Storage of freshly collected material for two years resulted in the disappearance of all alkaloids except 5-OH-DMT. This may raise speculation as to whether or not they were originally contained in the Spruce material. Our observation stresses the importance of storage-time in addition to knowledge of plant part, soil, season and climatic conditions, when alkaloid analysis is carried out on seeds and on the snuffs prepared from them.

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PLATE 61

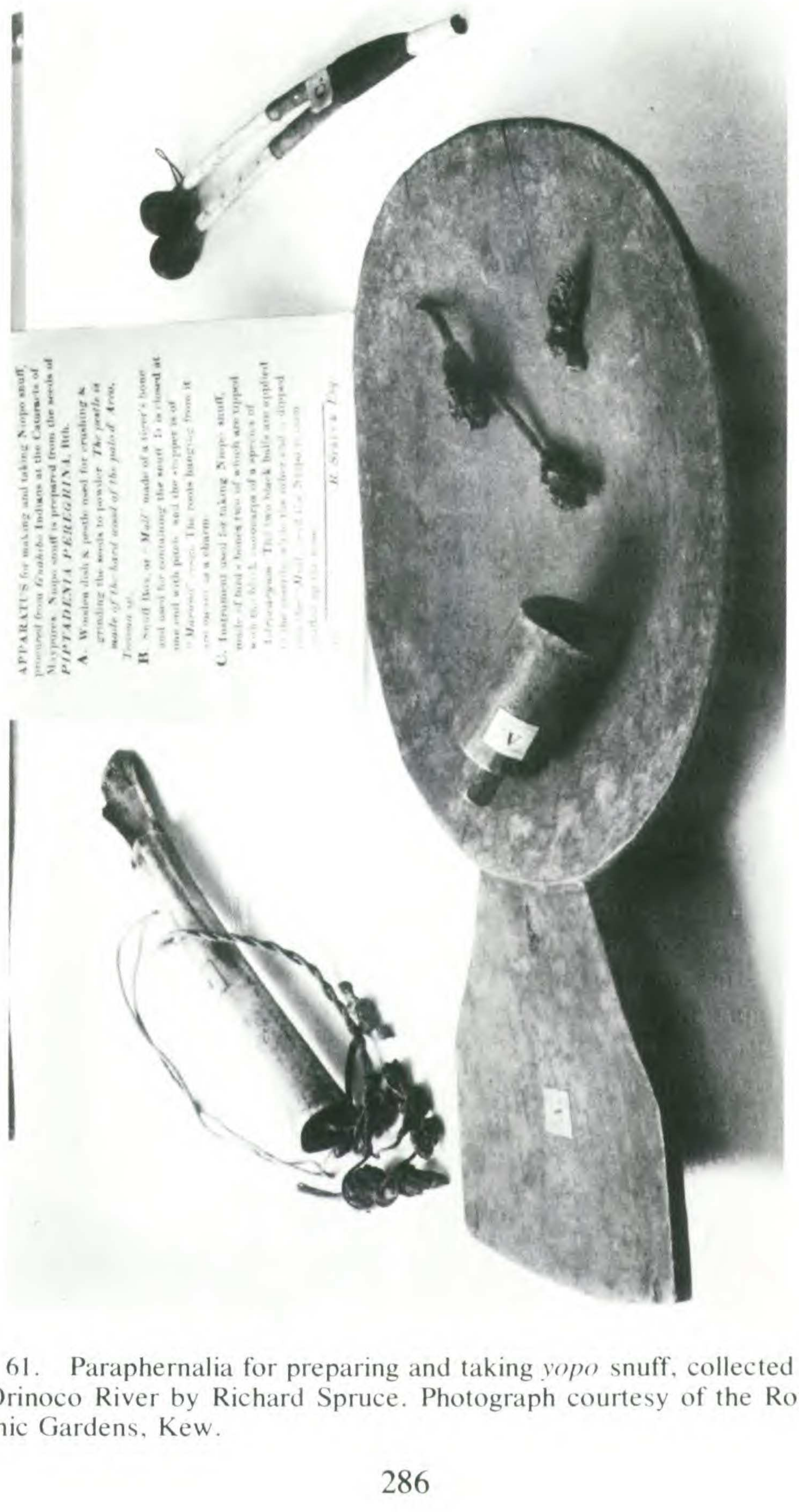

Plate 61. Paraphernalia for preparing and taking *yopo* snuff, collected on the Orinoco River by Richard Spruce. Photograph courtesy of the Royal Botanic Gardens, Kew.

PLATE 62

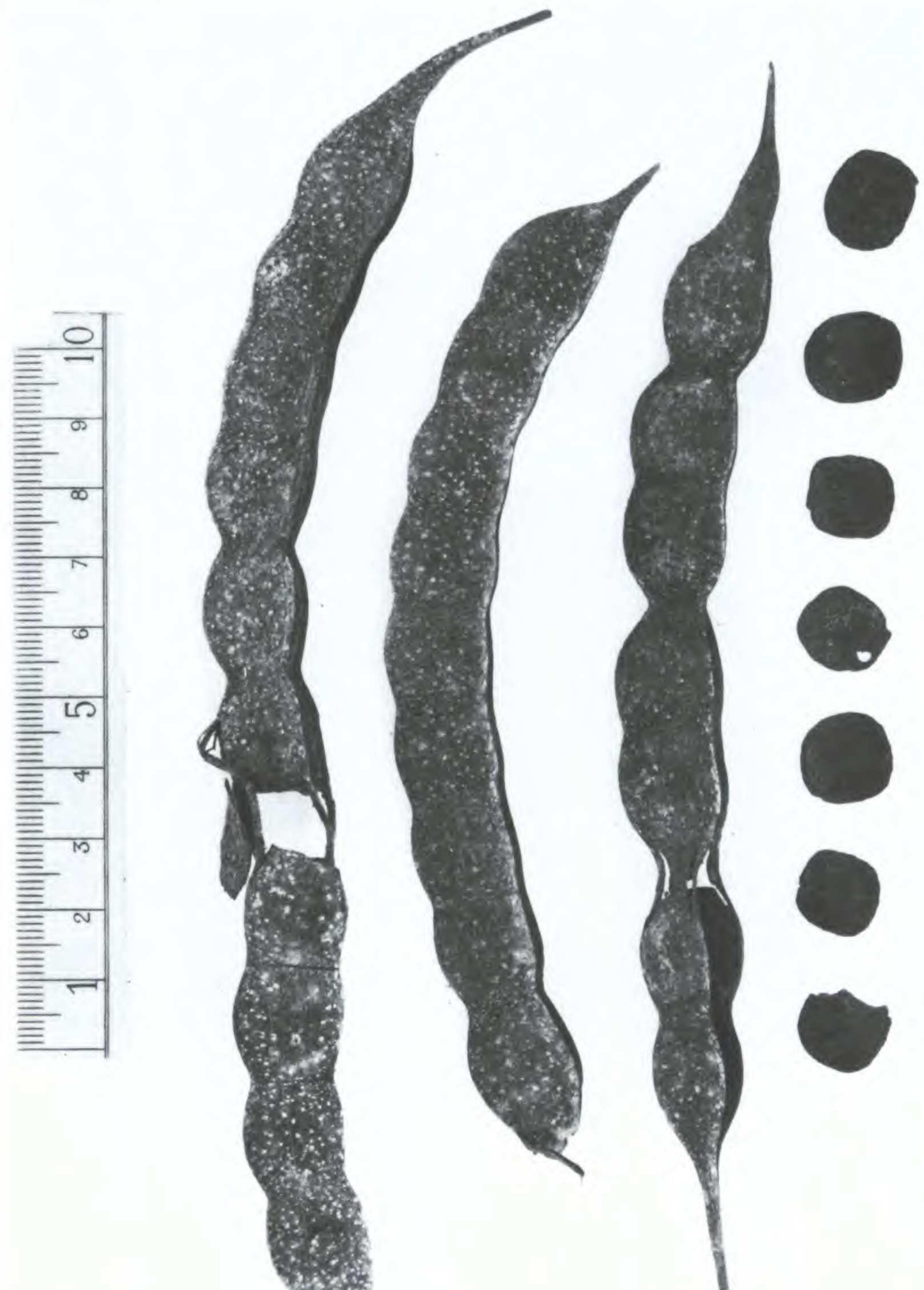

Plate 62. Pods and beans of *Anadenanthera peregrina* collected on the Orinoco River in 1854 by Richard Spruce. Photograph courtesy of the Royal Botanic Gardens, Kew.